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Prevalence of human astrovirus serotypes in the Oxford region 1976–92, with evidence for two new serotypes

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SUMMARY

Results of serotyping on 291 astrovirus-positive stools collected between 1976 and 1992 showed that about two-thirds (64·9%) were serotype 1. Infections were more frequent in the fourth quarter of the year and there was a suggestion that during the past 5 years serotype 1 has occurred with greater frequency in alternate years. Evidence is provided for the existence of two new serotypes, 6 and 7.

INTRODUCTION

Astroviruses are now clearly established as one of the common causes of gastroenteritis in young children and, less commonly, in adults [1–4]. In a survey of symptomless children and young adults in the UK, 75% of those aged 5–10 years and 77% aged 17–30 were shown to have astrovirus antibody, confirming its role in common childhood infections [5].

In Bangkok (Thailand), Herrmann and colleagues [6] found astroviruses in 8·6% of children under 5 years attending an out-patient clinic with gastroenteritis, but in only 2% of symptomless, but otherwise matched, children. Astroviruses were found more frequently than enteric adenoviruses but only half as often as rotaviruses. In a study of children 0–3 years old in a rural community in Guatemala, 7·3% of diarrhoea episodes were associated with astrovirus [7]. Nazer and colleagues [8] found that 16 of 28 children who had astrovirus-associated gastroenteritis in a children's hospital in London were also excreting other recognized bacterial or viral pathogens, most frequently rotaviruses (40%).

Astroviruses cannot readily be propagated in routine tissue culture systems and diagnosis is at present largely dependent on visualization of the virus in the electron microscope. More recently, enzyme immunoassay [9], dot blot hybridization [10] and the polymerase chain reaction [11] have also been shown to be useful diagnostic procedures.

We have previously reported the existence of five serotypes of human astrovirus based on an immunofluorescence test (IFT) [12] and immunosorbent electron microscopy (ISEM) [13] using hyperimmune rabbit antisera. With these methods, 73 astroviruses, 69 locally acquired, were assigned to their respective types [14].

We now report the occurrence of two further serotypes. Serotype 6 was found in June 1989 in a diarrhoeic stool specimen from an 11-month-old female in a

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paediatric ward at the John Radcliffe Hospital, Oxford. Serotype 7 has been detected twice; first, in April 1991, from a 1-year-old male who had diarrhoea and attended an Oxford practice, and more recently from a 19-year-old with severe diarrhoea on returning from Central America.

We also provide an update on the serotyping of 291 astrovirus strains found in stool specimens received between 1976–92 from patients in the Oxford region, either hospitalized or visiting their general practitioners.

MATERIALS AND METHODS

Specimens

Stool specimens received at the Oxford Public Health Laboratory from children under 6 years of age with acute or recent gastroenteritis, and occasionally adults when a possible virus gastroenteritis was suspected, were examined for viruses by electron microscopy.

Treatment of faeces for electron microscopy

A 10% extract of faeces was prepared in phosphate-buffered saline (PBS) at pH 7·3 and clarified by centrifugation at 2000 g for 30 min. The supernatant was further centrifuged at 106500 g for 1 h. After draining, the pellet was resuspended in 100 μl of distilled water containing 100 μg/ml of bacitracin.

Formvar/carbon coated grids were floated onto the concentrates and left for 10 min. On removal, the grids were drained and allowed to dry partly before washing in distilled water and staining with 1% methylamine tungstate. They were examined in a Philips 301 transmission microscope at a magnification of $\times$40000.

Astrovirus rabbit antisera

The preparation of antisera against types 6 and 7 astroviruses used for IFT, ISEM and immune electron microscopy (IEM) was carried out as previously described [15] except that CaCo-2 cells were used to propagate the viruses for rabbit immunization.

Immune electron microscopy (IEM)

For routine serotyping of astroviruses, IEM was carried out by mixing 4 μl of a 1:100 dilution of rabbit antiserum with 4 μl of virus suspension (faecal extract, resuspended pellet, or medium from infected CaCo-2 cells) on a small piece of Benchkote (Whatman). This was incubated in a moist chamber at 35 °C for 1 h. During this period, formvar/carbon coated grids were placed onto drops of Protein A solution (10 μg/ml in PBS, Sigma) and left at room temperature for 20 min. They were then drained, rinsed three times in distilled water and placed onto the virus/antiserum mixture. After 10 min adsorption at room temperature, the grids were again washed three times in distilled water, stained with 1% methylamine tungstate and examined in the electron microscope.

RESULTS

Astrovirus types 6 and 7

In 1989 and 1991, two astroviruses were found which did not react with antisera to types 1–5. Both had the typical features of astroviruses when examined by

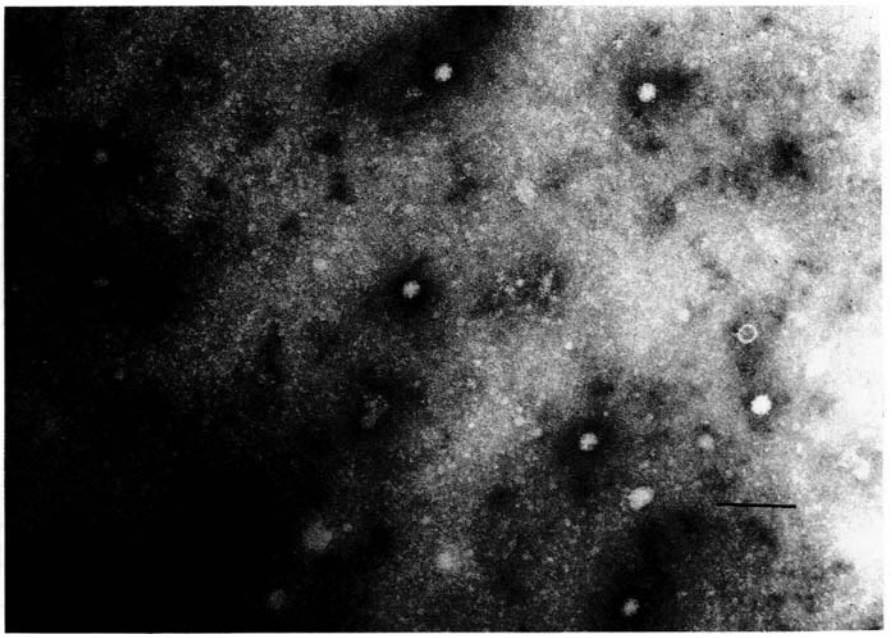

Fig. 1. Immunosorbent electron microscopy (ISEM) of astrovirus type 6, fifth passage in CaCo-2 cells (Bar, 100 nm).

Table 1. *Immunofluorescent antibody titres to astrovirus types 6 and 7 of type specific rabbit anti-astrovirus Sera (IFT)*

	Anti-astrovirus serum to type						
	1	2	3	4	5	6	7
Astrovirus type 6	< 20	< 20	< 20	< 20	< 20	640	< 80*
Astrovirus type 7	< 20	< 20	< 20	< 20	< 20	< 80*	320

* High level of non-specific background fluorescence when tested at a dilution of 1:20.

Table 2. *Immunosorbent electron microscopy (ISEM) particle count with type-specific anti-astrovirus sera*

	Anti-astrovirus serum to type							
	1	2	3	4	5	6	7	C†
Astrovirus type 6	11*	4	13	67	24	1208	2	5
Astrovirus type 7	3	2	1	3	1	3	371	2

* Average number of virus particles/grid square.
† Control (non-coated grid).

electron microscopy (Fig. 1), replicated in CaCo-2 cells in the presence of trypsin, and reacted with a monoclonal antibody reactive with the other known astrovirus serotypes. Tables 1 and 2 show that these two viruses (putative types 6 and 7) did not cross-react with each other or with astrovirus types 1–5 when tested by IFT and ISEM. It was noted, however, that the background level of non-specific fluorescence of the two antisera prepared against astrovirus types 6 and 7 propagated in CaCo-2 cells was greater than that of antisera to types 1–5 which

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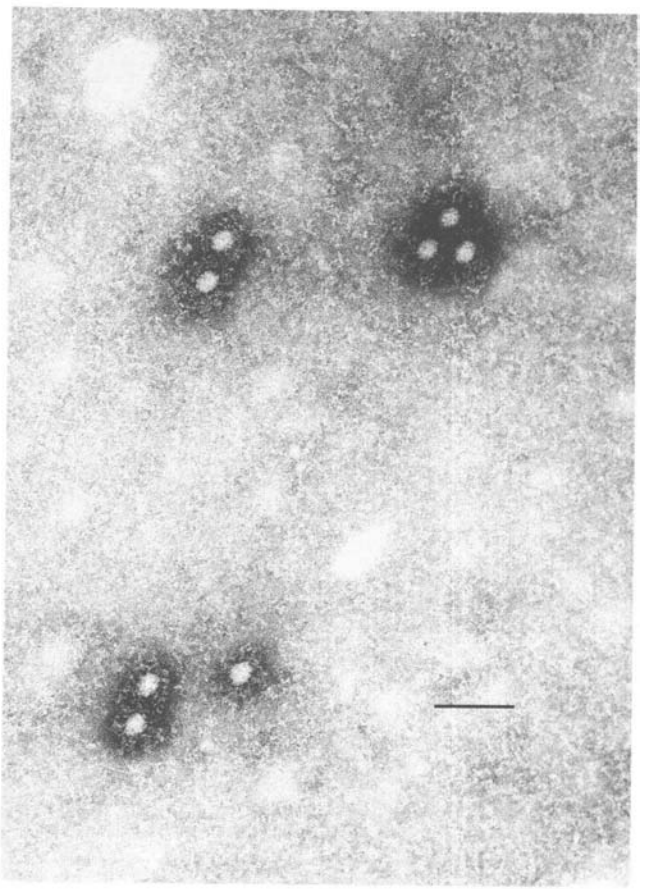

Fig. 2. Immune electron microscopy (IEM). Astrovirus type 1 virus coated with homologous rabbit anti-serum (Bar. 100 nm).

Table 3. *Serotyping of astroviruses in the Oxford region, 1976–92 (cumulative totals)*

	Serotype						
	1	2	3	4	5	6	7
Total (291)	189	33	27	33	6	1	2
Percentage	64·9	11·3	9·3	11·3	2·1	0·3	0·7

had been grown in LLCMK2 cells. The following year (1992) a second type 7 astrovirus was found, epidemiologically unrelated to the previous isolate. Type 6 and one of the type 7 viruses were from infants in the Oxfordshire area who had gastroenteritis. The second type 7 virus was detected in a 19-year-old female with severe diarrhoea who recently returned from travel in Guatemala and Mexico.

Serotypes in the Oxfordshire region 1976–92

Results of serotyping by IEM (Fig. 2) of the 291 astrovirus-positive stool specimens collected during the 17-year survey period are summarized in Table 3. In 15 of the stools other viruses (13 rotaviruses, 2 adenoviruses), which are known

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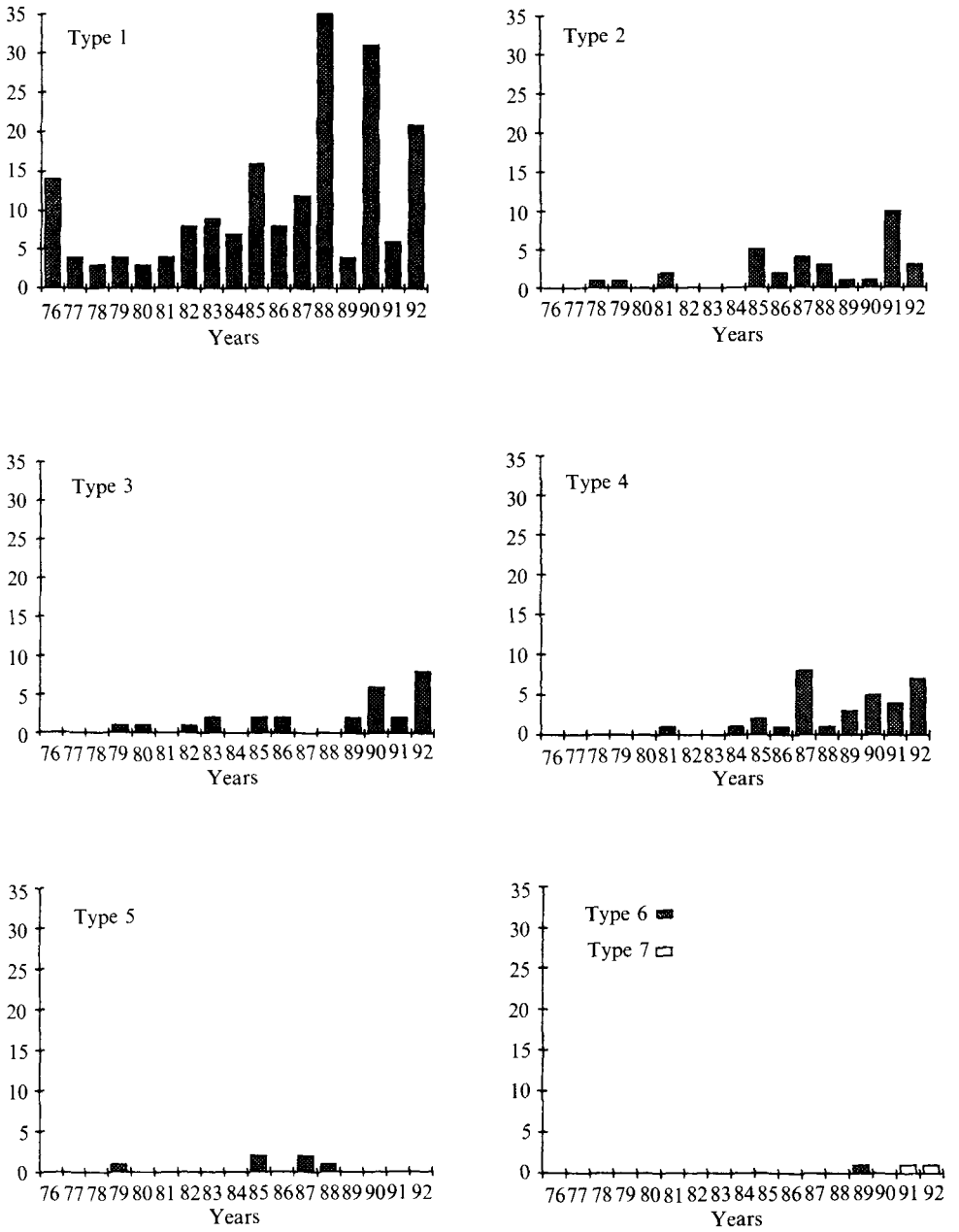

Fig. 3. Annual incidence of astrovirus serotypes 1–7 1976–92.

to be associated with gastroenteritis, were also present with astroviruses. Unfortunately no record of the presence of bacterial pathogens in these specimens is available. The prevalence of serotypes on a yearly basis is shown in Fig. 3. In each year of the survey, apart from 1991, astrovirus type 1 predominated, and overall, almost two thirds of all strains were found to be this serotype. When the figures for the paediatric wards at two Oxford hospitals were examined, the

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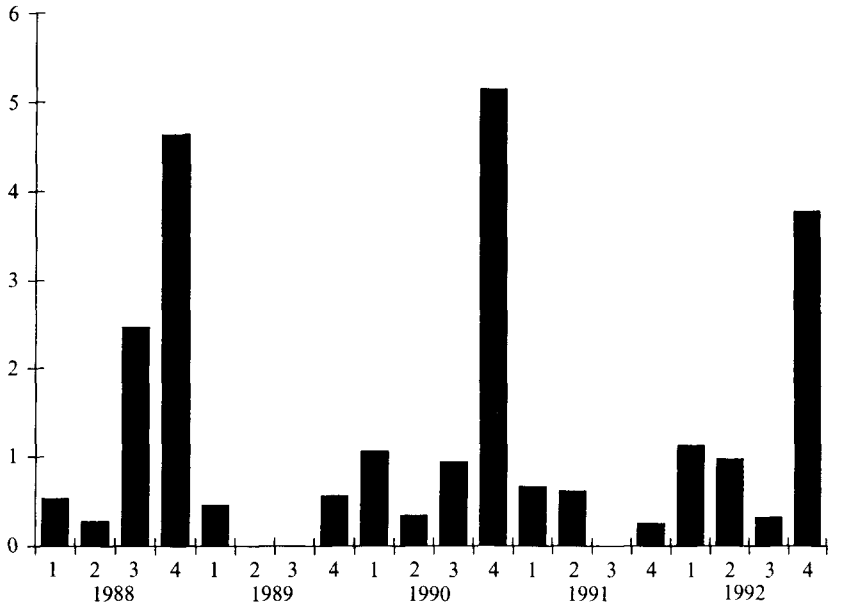

Fig. 4. Incidence of astrovirus serotype 1 per 100 faeces examined, by quarters. 1988–92.

prevalence of serotype 1 was 77·2% compared to a prevalence of 59·9% in specimens sent to the laboratory from general practitioners. We noted biennial 'peaks' of serotype 1 over the last 5 years, the increased numbers occurring in 1988, 1990 and 1992 (Fig. 4). The comparatively low incidence of serotypes 2, 3 and 4 has remained fairly constant throughout the survey period. Serotype 5 virus was first discovered in 1979, was not observed again until 1985, and has not been seen in this locality since 1988.

DISCUSSION

We have previously reported the existence of five serotypes of astrovirus, discovered between 1976 and 81. There was then a period of 8 and 10 years respectively before type 6 and type 7 were found.

The incidence of serotype 1 was higher (77·2%) in the paediatric wards than in specimens sent from general practices (59·9%). The higher figure would almost certainly suggest nosocomial transmission whereas the lower one is a more reliable indicator of the prevalence of illness caused by this serotype in the community as a whole. In only one year, 1991, was serotype 1 not the most frequent, type 2 being found more often. The biennial 'peaks' of serotype 1 in 1988, 1990 and 1992, are something not previously observed and suggest that this serotype 1 is present in the community more commonly in alternate years.

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